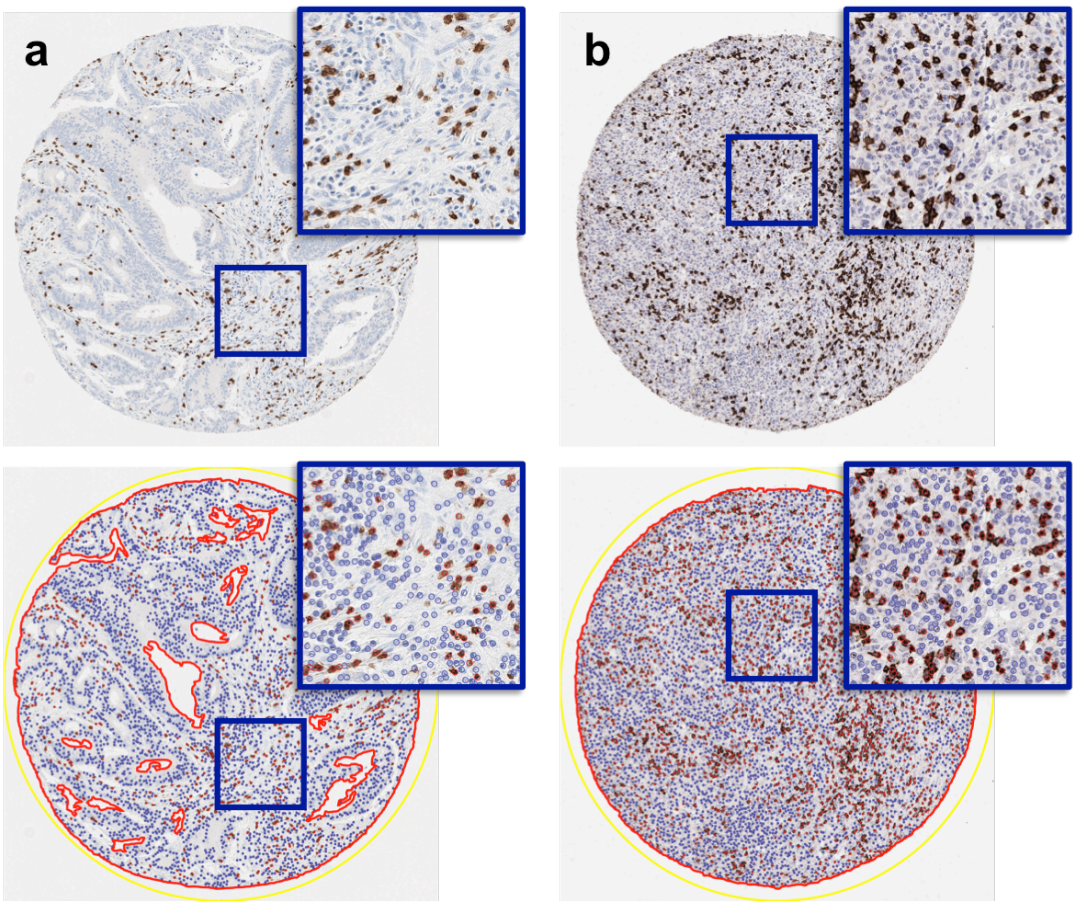
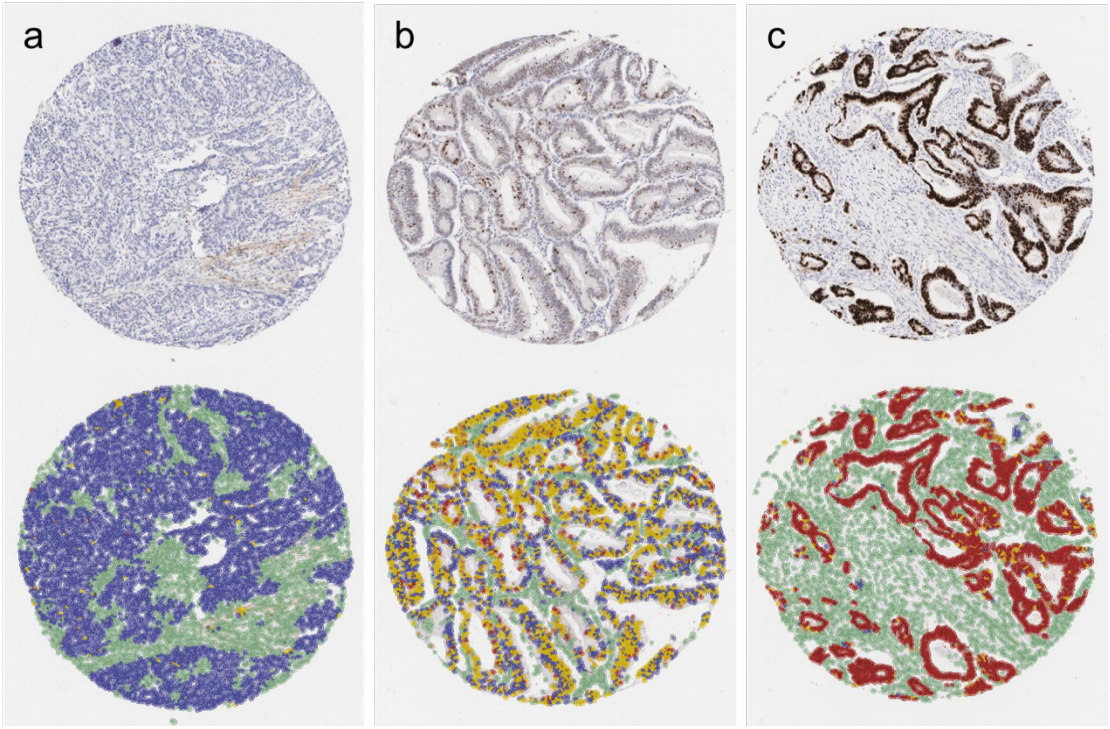


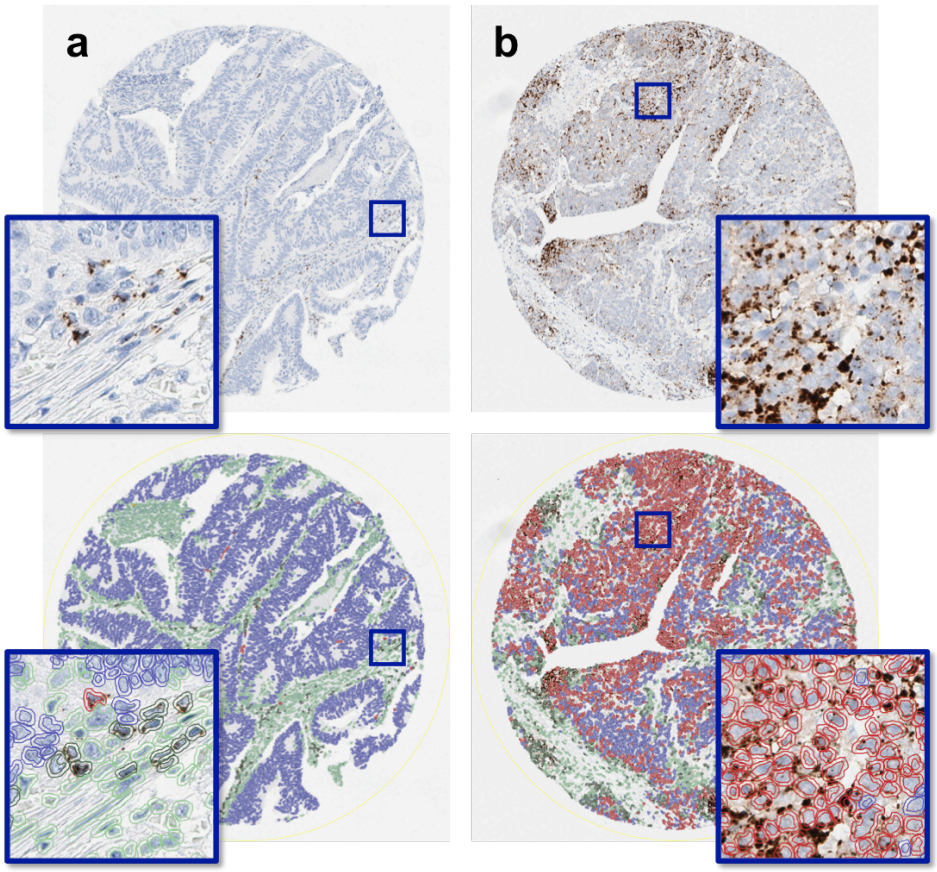
Supplementary Figure 1

Analysis of (**a**) CD3 and (**b**) CD8 as the number of positive cells per mm^2 tissue. The red boundary in each markup image denotes the detected tissue region. Detected positive cells are shown as small red circles. The total count of positive cells is divided by the tissue area to give the final output. Negative cells (blue) are detected, but do not contribute to the score.



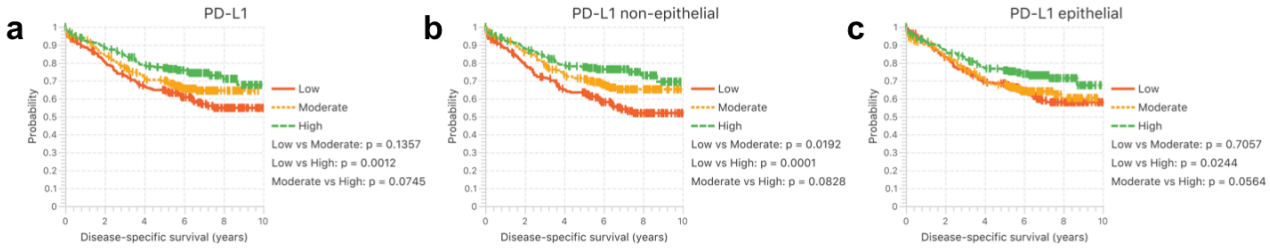
Supplementary Figure 2

Original and markup images of p53-stained TMA cores showing cell detection and classification for p53 immunoscoring using QuPath. Non-epithelial cells are shown in light green. Epithelial cells are colored according to p53 expression based on mean DAB nuclear staining intensity as follows: blue (negative), yellow (weak positive), orange (moderate positive) and red (strong positive). (**a**) Extreme negative p53 expression (H-score = 1.4). (**b**) Moderate p53 expression (H-score = 74.7). (**c**) Extreme positive p53 expression (H-score = 277.3).



Supplementary Figure 3

Original and markup images showing PD-L1 cell detection and classification using QuPath. Detected cells are color-coded according to classification: light green (non-epithelial, negative), dark green (non-epithelial, positive), blue (epithelial, negative) and red (epithelial, positive). (**a**) Moderate PD-L1 expression (2.9 % positive cells). (**b**) High PD-L1 expression (48.7 % positive cells).



Supplementary Figure 4

Survival analysis of PD-L1 based on tertiles. (**a**) A moderate dose-response effect is suggested when considering PD-L1 in all cells. Tertile cutoff values are 0.8% and 2.6% positive cells per core. (**b**) Considering only cells classified as non-epithelial, a more pronounced separation in survival curves is seen. Cutoff values are also increased to 1.4% and 4.3%, indicating that most cells expressing PD-L1 positivity are non-epithelial. (**c**) A decreased separation in survival curves is seen when considering PD-L1 expression only within cells classified as epithelial. A statistically significant separation between low-and high expression is still evident, but this is based on extremely low cutoff values of 0.23% and 0.85% positive cells, and should therefore be interpreted with caution.

Supplementary Video 1

Demonstrating TMA analysis of CD3 using QuPath. The primary steps of TMA dearraying, stain estimation, tissue detection and cell counting are initially performed interactively to identify suitable parameters. These step are logged, and can then be converted into a script for batch processing. Tissue cores can also be ranked by score to perform a fast visualization and quality assurance.

Supplementary Video 2

Demonstrating cell classification for p53 using QuPath. Measurements of each cell are made during segmentation. These can both be visualized in the form of interactive 'measurement maps' and also used to train up a machine learning classifier. Here, a classifier is rapidly trained to distinguish tumor cells and score each cell according to p53 expression. Training proceeds by using QuPath's drawing tools to select regions containing cells of different classes (here, red indicates tumor epithelium and green non-tumor or stroma), during which a random trees classifier is generated and applied across all cells within the slide to provide real-time feedback on classification performance. Intensity thresholds can also be set to generate appropriate summary scores, such as the H-score, which can be viewed and exported as required.